

NOTES

Significance of Protein A Production by Staphylococci

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Of 700 coagulase-positive staphylococcal strains isolated from humans, 692 (98.9%) produced protein A. Of 100 coagulase-negative strains, 2 were protein A-positive.

Coagulase production has been accepted as the primary criterion for differentiating pathogenic species of staphylococci (*Staphylococcus aureus*) from comensal strains (*S. albus*, *Bergey's Manual*, 7th ed.). There is a close correlation in staphylococci between deoxyribonuclease activity and coagulase production, and several authors recommend determination of deoxyribonuclease as another valuable criterion of the pathogenicity of the strains (1, 17).

Studies of staphylococcal protein A have indicated it to be a constituent of the cell wall (8, 14, 18) and, in addition, an extracellular product (4). Protein A reacts directly with the Fc portion of γ G-globulins of many mammalian species (3, 5, 6, 12). A direct reaction between the Fc portion of γ G-globulin and such a bacterial product is of considerable theoretical interest. It is possible that many events occurring in staphylococcal inflammatory reactions might be initiated by this γ G-protein A reaction (9, 10, 13, 16). Strains of *S. aureus* possessing large amounts of protein A tend to be more resistant to phagocytosis than strains that contain lesser amounts or none of this protein (2).

The purpose of the present work was to investigate the distribution of extracellular and cell-bound protein A in staphylococci and to correlate it to criteria of pathogenicity: deoxyribonuclease and coagulase production.

Pathogenic and apathogenic staphylococcal strains isolated from current human diagnostic specimens were obtained from several hospitals in Sweden. The strains were grown in tubes containing nutrient broth for about 10 hr under aeration by shaking to a concentration of 10^9 cells/ml, and the bacteria were harvested by centrifugation. Cell-bound protein A was quantitatively extracted by boiling suspensions of staphylococci in phosphate buffer, pH 5.9, for 1 hr (7). Extracellular

and cell-bound protein A were then separately in a series of doubling dilutions titrated by a hemagglutination technique, the principle of which is that protein A causes agglutination of sheep red blood cells sensitized with rabbit antibodies by reacting with the Fc part of the rabbit immunoglobulins (8, 16). The concentrations of protein A were determined by reference to standard preparations of cell-bound and extracellular protein A obtained from *S. aureus*, Cowan 1, and highly purified by diethylaminoethyl cellulose-Sephadex chromatography and gel filtration (7). In the hemagglutination test the specific activity was approximately 30 times higher for extracellular than for the heat-extracted cell-bound protein A. Coagulase was determined by using Coagulase Plasma (Difco) exactly according to the instructions provided by the manufacturer. Deoxyribonuclease activity was demonstrated on BBL deoxyribonuclease test agar (1).

Table 1 summarizes the results of the tests on 700 coagulase-positive and 100 coagulase-negative strains of staphylococci. Of 700 coagulase-positive strains, all were deoxyribonuclease-positive, and extracellular protein A was demonstrable in all but 8 strains. Of 100 coagulase-negative strains, two produced protein A and were also deoxyribonuclease-positive. Figure 1 demonstrates the quantitative distribution of extracellular protein A in the 700 coagulase-positive staphylococcal strains. The group of strains producing most protein A includes Cowan 1, which has mainly been used for studies of protein A. A comparable distribution spectrum was obtained for cell-bound protein A. On a weight basis, two to six times more cell-bound protein A than extracellular protein A was detected. However, in 20 of the strains producing extracellular protein A, cell-bound protein A could not be detected. As the specific activity in the hemagglutination test was approximately

30 times lower for purified, heat-extracted, cell-bound protein A than for purified extracellular protein A, it might be that very low contents of cell-bound protein A could not be detected with the technique used.

No detailed study of the distribution of protein A in strains of *S. aureus* has been published, but it was reported that nearly all strains of *S. aureus* encountered in routine work and all of 13 type strains except Wood 46 contain protein A (11, 14). In this study a small amount of extracellular as well as cell-bound protein was detected in Wood 46, probably because of the sensitivity of the hemagglutination test.

The data presented in Table 1 show good correlation between protein A production, deoxyribonuclease, and coagulase activity. The parallel observed between these apparently dissimilar properties warrants further investigation. Although no definite statement can yet be made about the role of protein A in staphylococcal pathogenicity, it is of interest that almost all strains regarded as potentially pathogenic produce this protein in addition to deoxyribonu-

clease and coagulase. The ability of staphylococci to produce protein A may be more closely linked to a mechanism of pathogenesis than its capacity to destroy deoxyribonucleic acid and to coagulate blood plasma. In conjunction with clinical and experimental studies, it should be of value to investigate the significance of protein A production in relation to the pathogenicity of staphylococci.

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LITERATURE CITED

1. Di Salvo, J. W. 1958. Desoxyribonuclease activity of micrococci. *Med. Tech. Bull.* 9:191-196.
2. Dosset, J. H., G. Kronvall, R. C. Williams, and P. G. Quie. 1969. Antiphagocytic effects of staphylococcal protein A. *J. Immunol.* 103:1405-1410.
3. Forsgren, A. 1968. Protein A from *Staphylococcus aureus*. VI. *J. Immunol.* 100:927-930.
4. Forsgren, A. 1969. Protein A from *Staphylococcus aureus*. VIII. *Acta Pathol. Microbiol. Scand.* 75:481-490.
5. Forsgren, A., and J. Sjöquist. 1966. "Protein A" from *S. aureus*. I. Pseudo-immune reaction with human γ globulin. *J. Immunol.* 97:822-827.
6. Forsgren, A., and J. Sjöquist. 1967. "Protein A" from *Staphylococcus aureus*. III. Reaction with rabbit γ -globulin. *J. Immunol.* 99:19-24.
7. Forsgren, A., and J. Sjöquist. 1969. Protein A from *Staphylococcus aureus*. VII. Physicochemical and immunological characterization of protein A. *Acta Pathol. Microbiol. Scand.* 75:466-480.
8. Forsgren, A., and U. Forsum. 1970. Role of protein A in nonspecific immunofluorescence of *Staphylococcus aureus*. *Infect. Immun.* 2:387-391.
9. Gustafson, G. T., J. Sjöquist, and G. Stålenheim. 1967. "Protein A" from *Staphylococcus aureus*. II. *J. Immunol.* 98:1178-1181.
10. Gustafson, G. T., G. Stålenheim, A. Forsgren, and J. Sjöquist. 1968. "Protein A" from *Staphylococcus aureus*. IV. *J. Immunol.* 100:530-534.
11. Kronvall, G., P. G. Quie, and R. C. Williams. 1970. Quantitation of staphylococcal protein A: determination of equilibrium constant and number of protein A residues on bacteria. *J. Immunol.* 104:273-278.
12. Kronvall, G., U. S. Seal, J. Finstad, and R. C. Williams. 1970. Phylogenetic insight into evolution of mammalian Fc fragment of γ G-globulin using staphylococcal protein A. *J. Immunol.* 104:140-147.
13. Martin, R. R., J. G. Crowder, and A. White. 1967. Human reactions to staphylococcal antigens. A possible role of lysosomal enzymes. *J. Immunol.* 99:269-275.
14. Nickerson, D. S., J. G. White, G. Kronvall, R. C. Williams, and P. G. Quie. 1970. Indirect visualization of *Staphylococcus aureus* protein A. *J. Exp. Med.* 131:1039-1047.
15. Oeding, P., and G. Haukenes. 1963. Identification of *Staphylococcus aureus* antigens and antibodies by means of the gel precipitation technique. *Acta Pathol. Microbiol. Scand.* 57:438-450.
16. Sjöquist, J., and G. Stålenheim. 1969. Protein A from *Staphylococcus aureus*. IX. *J. Immunol.* 103:467-473.
17. Weckman, B. G., and B. W. Catlin. 1957. Deoxyribonuclease activity of micrococci from clinical sources. *J. Bacteriol.* 73:747-753.
18. Yoshida, A., S. Mudd, and N. A. Lenhart. 1963. The common protein agglutinin of *Staphylococcus aureus*. II. Purification, chemical characterization, and serologic comparison with Jensen's antigen. *J. Immunol.* 91:777-782.

TABLE 1. Incidence of protein A and deoxyribonuclease production by coagulase-positive and coagulase-negative staphylococci

Staphylococci	No. of strains	Deoxyribonuclease-positive	Extracellular protein A production		
			Positive	Negative	Positive %
Coagulase-positive	700	700	692	8	98.9
Coagulase-negative	100	2	2	98	2.0

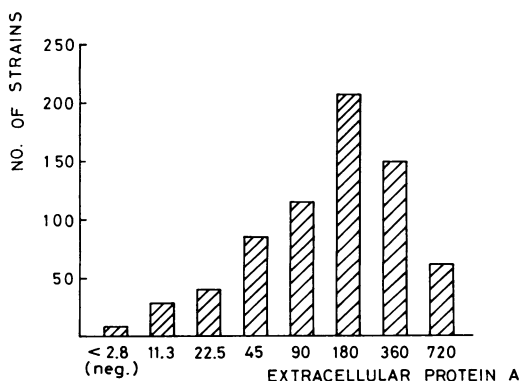


FIG. 1. Extracellular protein A (in nanograms) produced in 1 ml of nutrient broth by 10^8 bacteria of 700 different coagulase-positive staphylococcal strains. Extracellular protein A given in nanograms per milliliter.